IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application Number	09/438,392
	Filing Date	12 November 1999
	First Named Inventor	Takashi AOYAMA
	Group Art Unit	1635
	Examiner Name	Jane Zara
	Attorney Docket Number	2312-105
Title of the Invention: CHEMICAL INDUCIBLE PROMOTER USED TO OBTAIN TRANSGENIC PLANTS WITH A SILENT MARKER		

COMMUNICATION AND TRANSMITTAL OF DECLARATION UNDER RULE 132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Further to the Response to Office Action filed on 11 August 2003, Applicants submit a Declaration Under Rule 132 by Dr. Nam-Hai Chua to further support the arguments set forth in that response.

Specifically, the present claims recite a DNA sequence encoding a transcription factor that has (1) a promoter, (2) DNA encoding a DNA binding domain of the bacterial repressor LexA, (3) DNA encoding a transactivating domain of VP16 and (4) DNA encoding the regulatory domain of an estrogen receptor (ER), arranged in this order in the 5' to 3' direction. This transcription factor is called the "XVE system" herein. See Paragraph 4 of Dr. Chua's Declaration Under Rule 132.

The primary reference, Louvion et al., discloses (1) a transcription factor having a promoter, the DNA binding domain of GAL4, the transactivating domain of VP16 and the regulatory domain of the estrogen receptor (ER), called the "GVE system" herein and (2) transcription factor having a promoter, the DNA binding domain of GAL4, the regulatory domain of ER and the transactivating domain of VP16, called the "GEV system" herein. See Paragraph 7 Dr. Chua's Declaration Under Rule 132.

Although the primary reference discloses these two transcription factors, it discloses that the GVE system and the GEV system are not equivalent. See Paragraph 8 of Dr. Chua's Declaration Under Rule 132. Specifically, the Examiner's attention is directed to Table 1 on page 131 of Louvion et al. Table 1 demonstrates that the GEV system is able to induce βGal activity in KY320 cells by 202 fold and to induce βGal activity in GGY1::171 cells by 126 fold. In comparison, the GVE system is able to only induce βGal activity by 17 fold and by 24 fold, respectively. The GVE system does not result in as tight control of transcription regulation as the GEV system. Thus, Louvion et al. teaches a skilled artisan that the GEV system is vastly superior to the GVE system.

Although Goff et al. may suggest that the DNA binding domains of GAL4 and LexA may be interchangeable, Goff et al. does not describe any comparison of the activities of these DNA binding domains in the system of Goff et al, and thus does not provide any teaching as to what reasonable expectation of success may be with respect to the activity of these binding domains in a transcriptional activator. See Paragraph 9 of Dr. Chua's Declaration Under Rule 132. Furthermore, Goff et al. does not even describe the same gene expression controlling system as either Louvion et al. or the present application. Thus, a skilled artisan would not gain any guidance from Goff et al. to arrive at the present invention, and in fact the results achieved with the present invention are at odds with Goff et al. See Paragraphs 10 and 11 of Dr. Chua's Declaration Under Rule 132.

In view of these facts, it is Dr. Chua's opinion that the teachings of Goff et al. would not give a skilled artisan a sufficient expectation of success for substituting Lex A for Gal4 in the system of Louvion et al., which is different than the system of Goff et al. Since the gene control systems of these references are different, it is further Dr. Chua's opinion that there would be no motivation to combine the references. Furthermore, there is no suggestion in Louvion et al. and Goff et al. that substituting LexA for Gal4 in the loose regulatory system of Louvion et al. would produce a system with tight regulation. See Paragraph 12 and 13 of Dr. Chua's Declaration Under Rule 132.

In addition to these opinions of Dr. Chua, the Declaration Under Rule 132 describes the significant unexpected results that are achieved by the present invention that are not achieved with prior art systems. With the XVE system it is possible to consistently achieve 100-200 fold enhancement of gene expression. This result is in contrast, the highest level of induction achieved

with Louvion et al.'s GVE system which was 17-24 fold. See Paragraph 14 of Dr. Chua's Declaration Under Rule 132. There is no teaching in the prior art that would suggest the results achieved by the claimed invention. See Paragraph 15 of Dr. Chua's Declaration Under Rule 132.

Thus, it is submitted that not only is there no motivation in the cited prior art to arrive at the presently claimed invention, the presently claimed invention has unexpected results over the Examiner's proposed substitution of LexA for Gal4. Therefore, it is submitted that the claims are not obvious over the cited references.

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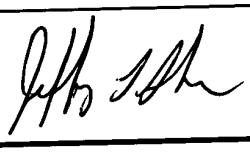
<tr><td colspan="6">RESPECTFULLY SUBMITTED,</td></tr>
<tr><td>Name and Reg. Number</td><td colspan="5">Jeffrey L. Ihnen, Reg. No. 28,957</td></tr>
<tr><td>Signature</td><td colspan="2">  </td><td>Date</td><td colspan="2">9 October 2003</td></tr>
<tr><td>Address</td><td colspan="5">Rothwell, Figg, Ernst & Manbeck
Suite 800, 1425 K Street, N.W.</td></tr>
<tr><td>City</td><td>Washington</td><td>State</td><td>D.C.</td><td>Zip Code</td><td>20005</td></tr>
<tr><td>Country</td><td>U.S.A.</td><td>Telephone</td><td>202-783-6040</td><td>Fax</td><td>202-783-6031</td></tr>
</table>

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DECLARATION UNDER RULE 132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Nam-Hai Chua, declare as follows:

1. I am the inventor of the subject application.

2. My education and experience are as follows. I received a Bachelor of Science degree in Botany and Biochemistry from the University of Singapore in 1965, a Masters degree in Biology from Harvard University in 1967 and a Doctorate degree in Biology from Harvard University in 1969. I have been employed by Rockefeller University, the assignee of the present application, from 1971 to present. From 1971 to 1988, I have been a Research Associate, Assistant Professor and Associate Professor in the Cell Biology Department and Professor and Head of the Laboratory of Plant Molecular Biology. Since 1988, I have been the Andrew W. Mellon, Professor and Head, Laboratory of Plant Molecular Biology. I have been involved in the area of transgenic plants, including plant transformation and regeneration since at least 1984.

3. I understand that the present claims recite a vector comprising a DNA sequence encoding a transcription factor that has (1) a promoter, (2) DNA encoding a DNA binding domain

of the bacterial repressor LexA, (3) DNA encoding a transactivating domain of VP16 and (4) DNA encoding the regulatory domain of an estrogen receptor (ER), arranged in this order in the 5' to 3' direction (claim 67). This vector produces a transcription factor that has the DNA binding domain of LexA, the transactivating domain of VP16 and the regulatory domain of the estrogen receptor (ER), arranged in this order in the amino to carboxy direction. This transcription factor is referred to as the "XVE system." Also claimed is an isolated nucleic acid comprising these elements (claim 78) and a transgenic plant or plant cell comprising the nucleic acid (claim 79).

5. I have recently reviewed this application and the Office Action mailed February 10, 2003. I have also recently reviewed the Louvion et al. (*Gene* 131:129-134, 1993) and Goff et al. (U.S. Patent No. 6,147,282) references cited in the Office Action. I am also familiar with the cited Aoyama et al. (*The Plant J* 11:605-612, 1997), Braselmann et al. (*Proc Natl Acad Sci USA* 90:1657-1661, 1993) and Schena et al. (*Proc Natl Acad Sci USA* 88:10421-10425, 1991), Draper et al. (U.S. Patent No. 6,031,151) and Kreppers et al. (U.S. Patent No. 5,880,331) references.

6. I understand that the Examiner has stated that the claimed invention is unpatentable because it would have been obvious to persons skilled in the art to "modify the vector of Louvion et al by exchanging the GAL4 binding domain with a lexA binding domain because the interchanging of these binding domains in transcription systems, and in combination with VP16, has been taught previously by Goff." Office Action at page 5. I also understand that the Examiner has argued that "[o]ne of ordinary skill in the art would have been motivated to utilize either GAL4 or lexA in combination with VP16 as a transcriptional activator because it had been taught previously that the swapping of response elements and binding domains provides increased flexibility in controlling gene expression" citing column 9, lines 16-32 of Goff et al. Office Action at pages 5-6. Finally, I understand that the Examiner has stated that "[o]ne of ordinary skill in the art [sic] would have expected to successfully utilize the combination of these components (e.g. either GAL4 or lexA in combination with VP16) in a transcription system because such routine exchange had been taught previously by others in the art, including Goff."

7. The primary reference, Louvion et al., discloses a transcription factor having a promoter, the DNA binding domain of GAL4, the transactivating domain of VP16 and the regulatory domain of the estrogen receptor (ER), arranged in this order in the amino to carboxy direction. This transcription factor is referred to as the "GVE system." This reference further discloses a transcription factor in which the transactivating domain of VP16 and the regulatory domain of ER are switched in their order within the transcription factor, i.e., a transcription factor having a promoter, the DNA binding domain of GAL4, the regulatory domain of the estrogen receptor (ER) and the transactivating domain of VP16, arranged in this order in the amino to carboxy direction. This latter transcription factor is referred to as the "GEV system."

8. Although the primary reference discloses these two transcription factors, it specifically discloses that the GVE system and the GEV system are not equivalent. See, Table 1 on page 131 of Louvion et al. Table 1 demonstrates that the GEV system is able to induce _Gal activity in KY320 cells by 202 fold and to induce _Gal activity in GGY1::171 cells by 126 fold.[1] In comparison, the GVE system is able to only induce _Gal activity by 17 fold in KY320 cells and by 24 fold in GGY1::171 cells. According to Louvion et al., the GVE system did not have tight control of transcription regulation as was the case for the GEV system. For further studies, Louvion et al. focused on the GEV system because of its tighter regulation. See, page 132, right column, last sentence of Louvion et al. Thus, Louvion et al. teaches a skilled artisan that the GEV system is vastly superior to the GVE system and that they are not equivalent.

9. The passage at column 9 of Goff et al. cited by the Examiner suggests that additional flexibility in controlling gene expression may be obtained by using DNA binding domains and response elements from other transcriptional activators. Two transcriptional activators described are GAL4 and LexA. Goff et al. contains no comparison of the effects of the DNA binding domains

[1] This high level of induction in the GEV system is also shown by Braselmann et al. which shows a 100-fold induction of activity in mammalian cells.

of these two transcriptional activators in the system of Goff et al., which is not the same system as described in Louvion et al. or the present invention, as described in further detail below. Since Goff et al. provides no comparison of these elements, there is no teaching as to what a reasonable expectation may be with respect to the activity of the corresponding factors.

10. As noted in Paragraph 9, Goff et al. does not describe the same gene expression controlling system as either the primary reference Louvion et al. or the present invention. Specifically, Goff et al. teaches a heterodimeric control system, where two different receptor polypeptides together activate gene transcription. See column 2, lines 55-65 and Figures 1-3. When identical receptor polypeptides are used in the Goff et al. system to form a homodimer, the result is gene repression, not activation. See column 10, lines 13-48. Louvion et al. and the present invention are directed to a homodimeric system, not the heterodimeric system of Goff et al. In the homodimeric systems of Louvion et al. and the present invention, gene activation is achieved and not repression as taught by Goff et al. Since the present invention is directed to an entirely different gene control system, a person of ordinary skill in the art would not draw particular guidance from the teachings of Goff et al. to arrive at the present invention.

11. Furthermore, the present invention, directed to a homodimeric system that achieved tight control of gene activation, is at complete odds with the teachings of Goff et al. With the system of the present claims, 100-200 fold induction of transcription is routinely achieved. See Examples 12-13 of the present application, and particularly page 30, lines 10-14. Goff et al., on the other hand, specifically states that Class 1 receptor polypeptides, such as the estrogen receptor recited in the present claims, do not function to activate gene expression. See column 1, line 60 - column 2, line 12 of Goff et al.

12. It is my opinion that the alleged teaching in Goff et al. of the interchangeability of the GAL4 and LexA DNA binding domains in a heterodimeric system for gene activation (or a homodimeric system for gene repression) would not give a person of ordinary skill in the art a sufficient expectation of success in substituting one for the other in a homodimeric gene activation

system as that described in Louvion et al. and the present claims. It is also my opinion that there is no motivation to combine Louvion et al. and Goff et al., since they are directed to different gene control systems.

13. Even if there was a motivation to modify the gene control system of Louvion et al. by substituting the LexA DNA binding domain for the GAL4 DNA binding domain of Louvion et al., such a substitution would not arrive at the invention as set forth in the present claims. As described in Paragraph 8 above, Louvion et al. teaches that the GEV system has tighter control of gene activation and that the GEV system is not equivalent to the GVE system. The substitution of the LexA DNA binding domain for the GAL4 binding domain in the GEV system would result in an XEV system, not the system of the present claims, i.e., the XVE system. There is also no teaching in either Louvion et al. or Goff et al. to lead a person of ordinary skill in the art to reasonably expect or predict that tight regulation may be achieved using a gene control system having loose regulation but which has a substitution of the LexA DNA binding domain for the GAL4 binding domain. That is, there are no teachings in the art cited by the Examiner, which would lead a person of ordinary skill in the art to reasonably expect that the substitution of the LexA DNA binding domain for the GAL4 DNA binding domain in the loose regulatory GVE system of Louvion et al. would produce a system with tight regulation.

14. As demonstrated by the present application, significant unexpected results are achieved by the present invention that are not achieved with prior art systems. Specifically, it is possible to consistently achieve 100-200 fold enhancement of gene expression with the XVE system. See Examples 12 and 13 of the present specification, especially page 30. In contrast, the highest level of induction achieved with Louvion et al.=s GVE system was 17-24 fold. See, Table 1, page 131 of Louvion et al.

15. There are no teachings in the art that the substitution of the DNA binding domain of LexA for the DNA binding domain of Gal4 would result in a transcription factor having a higher activity. In fact, the prior art (Goff et al.), as acknowledged by the Examiner, merely discloses an

interchangeability of LexA and Gal4. Thus, a skilled artisan would only expect an in-kind activity of the transcription factor with the Examiner=s proposed substitution in view of the teachings of the prior art, i.e., a 17-24 fold increase in gene expression. In contrast to this expectation by a skilled artisan, the present invention achieved a 100-200 fold enhancement of gene expression. This enhancement is a factor of 4.2 -11.8 times better than the GVE system of Louvion et al. It is my opinion that these results are not expected on the basis of the teachings of the art and clearly show the unexpectedness and the non-obvious nature of the present invention.

16. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or of any patent issued thereon.

Sept 12, 03
Date

Nam-Hai Chua

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